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Energetics of B-to-Z transition in DNA

(DNA supercoiling/DNA helical periodicity/two-dimensional electrophoresis/statistical mechanical treatment/structure in solution)

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ABSTRACT **Analysis by two-dimensional gel electrophoresis of topoisomers of plasmids containing $d(pCpG)_n \cdot d(pCpG)_n$ inserts, in which n ranges between 8 and 21, shows that the B-to-Z transition within the alternating C-G is readily induced by negative supercoiling and is highly cooperative. The free energy parameters for the transition in dilute aqueous buffers have been evaluated from a statistical mechanical analysis of the data, and these parameters allow prediction of the superhelicities of plasmids at which the transition occurs in alternating C-G inserts over a wide range of lengths. In agreement with the crystal structures, the helical handedness of the B structure in solution and that of the Z structure are shown to be opposite to each other. Furthermore, it is found that the B form of the alternating C-G sequence in solution has a helical periodicity of 10.5 ± 0.1 base pairs per turn, and the Z form has a helical periodicity of 11.6 ± 0.3 base pairs per turn. There also appears to be a significant unwinding of the right-handed DNA duplex at each of the B/Z junctions.**

Extensive studies of the left-handed Z-DNA structure have been carried out since the determination of this structure by Rich and co-workers (for recent reviews, see refs. 1–3). The energetics of the interconversion between the left-handed Z form and the right-handed B form under physiological conditions, however, has so far received only cursory attention (4). In the present communication, we report a detailed analysis of the dependence of the B-to-Z transition on DNA supercoiling. A set of plasmids containing $d(pCpG)_n \cdot d(pCpG)_n$ inserts, in which $n = 8$, 12, 16, and 21, has been constructed. For each plasmid, two-dimensional agarose gel electrophoresis has been used to examine simultaneously the mobilities of a group of topological isomers (topoisomers) that differ only in their linking numbers. The extent of the B-to-Z transition of the alternating C-G insert in each of the topoisomers can be measured from the two-dimensional electrophoretic patterns. A statistical mechanical analysis of the average number of base pairs that have flipped from the right-handed B to the left-handed Z structure as a function of linking number has enabled us to determine the free energy parameters for this transition. We have also applied the band-shift method (5, 6) to study the helical structure of the alternating C-G inserts; the results indicate that negative supercoiling of the DNA changes the alternating C-G sequence from a right-handed helix with 10.5 base pairs (bp) per turn to a left-handed helix with 11.6 bp per turn under physiological conditions.

MATERIALS AND METHODS

Plasmid Construction. Inserting 32 bp of alternating d(pCpG) into the filled-in *Bam*HI site of pBR322 to generate the plasmid pLP32 has been described (7). The plasmid pLP332 was constructed by inserting the *Bam*HI fragment containing the alternating C-G sequence into the *Bam*HI site of pTR161 (8). Plasmids pLP316, pLP324, and pLP342 were constructed by direct ligation of the phosphorylated octamer $d(pCpG)_4$ into the filled-in *Bam*HI site of pTR161. The nucleotide sequences of the appropriate insert-containing restriction fragments were determined according to the procedures of Maxam and Gilbert (9).

DNA topoisomers containing different linking numbers were prepared by relaxation of the DNA with calf thymus topoisomerase I in the presence of various amounts of ethidium bromide as described (6). Several samples with different ranges of linking numbers were pooled to give a mixture with a broader distribution of topoisomers.

Electrophoresis. Agarose gel electrophoresis was performed in horizontal gel slabs immersed in the electrophoresis buffer. For two-dimensional electrophoresis, 500 ml of melted agarose in TBE buffer (90 mM Tris·boric acid, pH 8.3/2.5 mM Na_2EDTA) was cast over a 1-foot-square (930-cm^2) glass plate with laboratory tape around its edges. Two 1/16-inch-square (0.16-cm^2) sample wells, 6 cm apart, were formed with a Delrin template that was held parallel to the top edge of the gel. Six microliters of a sample, containing a total of about 0.5 μg of DNA topoisomers plus 3% Ficoll and approximate amounts of tracking dyes, was loaded in each well through a glass micropipette with a flame-drawn tip. Electrophoresis in the first dimension was typically 20 hr at 110 V for a 0.7% gel. The gel was then soaked in a clean tray containing 2 liters of TBE plus chloroquine (10); equilibration takes 6–8 hr with gentle agitation. The final concentration of chloroquine was about 1.3 μM for the gels shown. After soaking, the buffer was transferred into the electrophoresis tank, and the gel was turned 90° from its original position. Electrophoresis in the second dimension was typically 18 hr at 100 V for a 0.7% gel. The gel was then soaked in several changes of deionized water to remove the chloroquine. Staining in water plus ethidium bromide at 1 μg/ml for 2 hr was followed by extensive destaining in several changes of water before photographing.

A smaller 8-inch-square (413-cm^2) gel was used for gels run in TBE plus either sodium chloride or hexammine cobalt(III) chloride. The electrophoresis buffer was recirculated through a 4-liter external buffer reservoir. In addition, the gel was cooled by running tap water underneath the Plexiglas platform under the gel. The electrophoresis voltage for 1.7% gels was about twice as high as that for 0.7% gels.

RESULTS

Dependence of the B-to-Z Transition on Supercoiling as Revealed by Two-Dimensional Gel Electrophoresis. Resolution of DNA topoisomers of different linking numbers by two-dimensional gel electrophoresis (11) was carried out by first running a mixture of topoisomers along one edge of a slab agar-

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Abbreviation: bp, base pair(s).

ose gel. Electrophoresis in the second dimension was carried out after soaking the gel in the electrophoresis buffer containing an appropriate concentration of chloroquine (10). Fig. 1 *Left* illustrates the results of such an experiment with a control plasmid pTR161. The topoisomers are resolved into distinct spots that lie along a smooth curve. As shown in Fig. 1 *Left*, the linking numbers of the spots decrease progressively as their enumeration increases: the linking number α of spot 6, for example, is lower than that of spot 5 by 1.

The two-dimensional analysis is particularly convenient for examining DNA supercoiling-induced structural changes. Fig. 1 *Right* illustrates the pattern obtained with plasmid pLP332, which is pTR161 containing a $d(pCpG)_{16}$·$d(pCpG)_{16}$ insert. The negative supercoiling-induced B-to-Z transition of the inserted alternating C-G sequence is revealed by a sharp break, around spot 17, in the curve that traces through the topoisomer spots. This break is due to the presence of base pairs in the left-handed helical conformation within the C-G insert in the more negatively supercoiled members of the mixture during electrophoresis in the first dimension. For a given negatively supercoiled topoisomer, the flipping of the helical hand of the C-G insert reduces its negative superhelicity and hence its gel electrophoretic mobility (4, 7, 12). Upon binding of chloroquine prior to second-dimension electrophoresis, the C-G insert in these topoisomers reverts to the right-handed structure, and thus the electrophoretic mobilities of all spots in the second dimension are similar to the corresponding ones of the control plasmid without the C-G insert. In order to facilitate comparison of the patterns shown in Fig. 1 *Left* and *Right*, the two mixtures were actually run simultaneously on the same slab gel by loading them into two wells separated by 6 cm.

Quantities That Can Be Measured from a Two-Dimensional Pattern. Two essential quantities for examining the details of a supercoiling-induced change in DNA helical structure can be evaluated directly from a two-dimensional gel pattern. The first quantity is the linking difference $\alpha - \alpha_0^\circ$ (13, 14) of each topoisomer, in which α_0° is the linking number of the DNA when it is completely relaxed under the first-dimension gel electrophoresis conditions. (For DNA rings that are smaller than a few hundred base pairs, the definition of α_0° needs modification because it deviates significantly from the average linking number of the completely relaxed DNA. This point will be discussed elsewhere. The linking difference of each topoisomer under the second-dimension conditions can also be evaluated, but this quantity is not needed here.) The apices of the two arcs indicated by arrows I and II in Fig. 1 *Left* correspond to the minima in electrophoretic mobilities in the first and second dimension, respectively. Apex I represents the position of a relaxed DNA under first-dimension electrophoresis conditions (in the TBE buffer at room temperature). The spots to the right of I are positively supercoiled during the first-dimension electrophoresis, whereas those to the left are negatively supercoiled. The quantity $\alpha - \alpha_0^\circ$ for spot 6, for example, is -2.5.

The second essential quantity that can be measured is the change in the twist number, ΔTw, when a transition, such as the B-to-Z transition studied here, occurs. As we have done previously (7), in the region where the topoisomers differ in their gel mobilities we assume that a pair of topoisomers with the same gel mobility has the same writhing number Wr. It then follows from the relationship $\alpha \equiv Lk = Tw + Wr$, in which Lk is the linking number (15, 16), that $\Delta Tw = \Delta\alpha$ for two topoisomers of the same Wr. The pair of spots 12 and 17 in Fig. 1 *Right*, for example, have about the same mobility and therefore $\Delta Tw = Tw_{17} - Tw_{12} = \alpha_{17} - \alpha_{12} = -5$. Actually, since the patterns in Fig. 1 *Left* and *Right* are obtained on the same gel, a straight line connecting the two nicked DNA spots in Fig. 1 serves as a reference line for comparing mobilities. With this reference line, the mobility of spot 17 in Fig. 1 *Right* interpolates between spots 12 and 13 at position 12.3, and the best estimate of $Tw_{17} - Tw_{12}$ is therefore -4.7.

The Supercoiling-Induced B-to-Z Transition Is Highly Cooperative. From Fig. 1 *Right* it is apparent that the supercoiling-induced B-to-Z transition occurs within a couple of turns of change in the linking number. A change of 1 in linking number corresponds to a change of 0.0024 in the specific linking difference or superhelical density (13) for the 4,400-bp plasmid used.

Intermediates of the sharp transition can be observed, however, by fine-tuning of the values of the linking differences $\alpha - \alpha_0^\circ$ of the topoisomers. One convenient way of such fine-tuning is by increasing or decreasing α_0° by a fraction of a turn by the insertion or deletion of a few base pairs that are not in

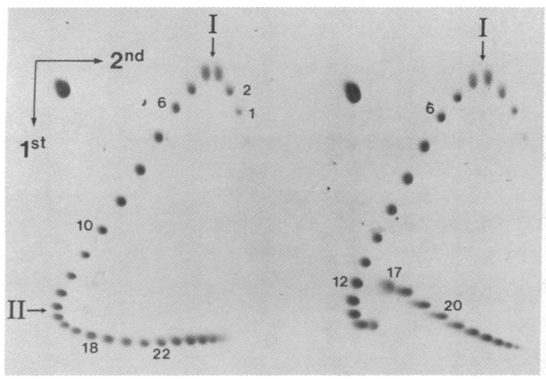

FIG. 1. Superhelicity dependence of the right- to left-handed conformational transition of cloned $d(pCpG)_{16}$·$d(pCpG)_{16}$ as revealed by two-dimensional agarose gel electrophoresis. The electrophoretic pattern of a mixture of topoisomers of plasmid pTR161 is shown in *Left* and that of the $d(pCpG)_{16}$-insert-containing plasmid pLP332 in *Right*. Both samples were electrophoresed in the same gel. Within a panel the topoisomers differ only in their linking number. The directions of electrophoresis are indicated in the figure. The buffer for the first dimension was 90 mM Tris·boric acid, pH 8.3/2.5 mM EDTA (TBE buffer). Prior to electrophoresis in the second dimension equilibration with TBE buffer containing 1.3 μM chloroquine unwound the plasmids about 12 turns. The dark spot in the upper left-hand corner of each panel corresponds to the nicked circular DNA.

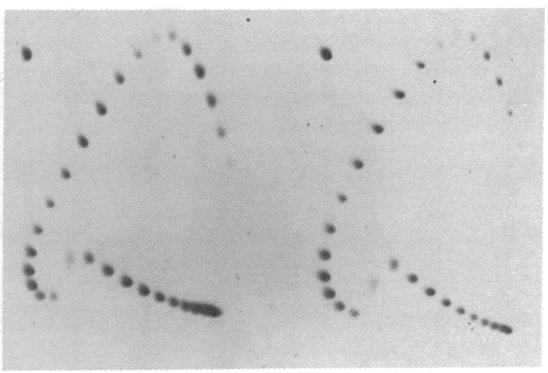

FIG. 2. The position of an intermediate of the B-to-Z transition is highly dependent on superhelicity. Both plasmid DNAs that give rise to the gel patterns shown contain a $d(pCpG)_{16}$ insert. *Right* corresponds to a mixture of topoisomers of plasmid pLP32. *Left* corresponds to a mixture of topoisomers of plasmid pLP32C, which is identical to pLP32 except that it is 2 bp longer because it has a filled-in *Cla* I site several hundred base pairs distant from the *Bam*HI site, where the insertion has been made.

the alternating C-G block. An increase of 2 bp in the length of the plasmid, for example, increases α_0° by about 0.2. Fig. 2 depicts the patterns when the length of the $d(pCpG)_{16}$·$d(pCpG)_{16}$ insert-containing plasmid is increased by 2 bp.

Dependence of the Supercoiling-Induced B-to-Z Transition on the Length of the Alternating C-G Insert. Two-dimensional gel electrophoresis was also carried out with plasmids pLP316, pLP324, and pLP342, which contain $d(pCpG)_n$·$d(pCpG)_n$ inserts with $n = 8$, 12, and 21, respectively. Data evaluated from all two-dimensional gel patterns are presented in Fig. 3.

Statistical Mechanical Modeling of the Supercoiling-Induced B-to-Z Transition and the Evaluation of the Free Energy Terms. We model the transition by assuming that within a block of $d(pCpG)_n$·$d(pCpG)_n$ there can be no more than a single block of i (pCpG·pCpG) units, with $i \leq n$. The free energy change when i is increased by 1 (pCpG·pCpG) unit is designated $2\Delta G_{BZ}$ when $i \neq 0$, and $2\Delta G_{BZ} + 2\Delta G_J$ when $i = 0$. ΔG_J is the free energy change for the generation of one B/Z junction. The partition function P_L for the B-to-Z interconversion within the alternating C-G block in a linear or nicked-circular DNA is therefore

$$P_L = 1 + \sum_{i=1}^{n} (n - i + 1)\,\sigma s^i, \qquad [1]$$

in which $\sigma \equiv \exp(-2\Delta G_J/RT)$ and $s \equiv \exp(-2\Delta G_{BZ}/RT)$, R and T being the gas constant and absolute temperature (17, 18).

When the B-to-Z interconversion occurs in a supercoiled DNA, the partition function P_s is

$$P_s = \exp[-K(\alpha - \alpha_0^\circ)^2/RT] + \sum_{i=1}^{n} (n - i + 1)\,\sigma s^i \exp[-K(\alpha - \alpha_0^\circ - ai - 2b)^2/RT], \qquad [2]$$

in which $K = 1{,}100\ RT/N$ for a DNA N bp long (N is several thousand bp or larger) (19, 20), and the term $ai + 2b$ is the total change in the helical twist when i changes from 0 to i. We take a to be $-2[(1/10.5) + (1/12)]$, which is the change in twist when 1 unit of pCpG·pCpG flips from a 10.5-bp-per-turn right-handed helix to a 12-bp-per-turn left-handed helix. The parameter b is included to account for any change in the helical twist at the B/Z junctions.

For a topoisomer molecule with a linking difference of $\alpha - \alpha_0^\circ$, the average change in twist due to the B-to-Z transition is

$$\langle \Delta Tw \rangle = \left\{ \sum_{i=1}^{n} (ai + 2b)\,(n - i + 1)\sigma s^i \times \exp[-K(\alpha - \alpha_0^\circ - ai - 2b)^2/RT] \right\} P_s^{-1} \qquad [3]$$

The measured changes in twist for topoisomers of different values of $\alpha - \alpha_0^\circ$ are fitted according to Eq. 3. A weighted nonlinear least-squares minimization with ΔG_{BZ}, ΔG_J, and b as independent parameters was performed. The curves drawn through the data points shown in Fig. 3 are those representing the best fit. The parameters used to obtain these curves are $\Delta G_{BZ} = +0.33$ kcal mol^{-1} per bp, $\Delta G_J = +5.0$ kcal mol^{-1} per junction, and $b = -0.4$ turn per junction (1 kcal = 4.184 kJ).

The determination of the parameters ΔG_{BZ}, ΔG_J, and b makes it possible to calculate the B-to-Z transition for $d(pCpG)_n$·$d(pCpG)_n$ inserts of any length. In Fig. 4, the specific linking difference at the midpoint of the transition is expressed as a function of $1/n$ (lower abscissa) or $2n$ (upper abscissa). The filled circles are data points from Fig. 3 and the solid line is the calculated function. It is clear from Fig. 4 that even for fairly small n the Z structure can form at physiological negative superhelicity. The meanings of the broken line and the open circle datum point will be given in a later section.

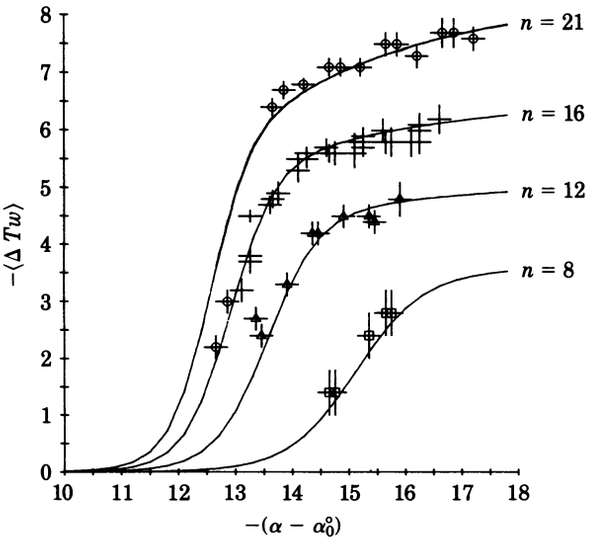

FIG. 3. Negative supercoiling-induced B-to-Z transition in plasmids containing $d(pCpG)_n$·$d(pCpG)_n$ inserts. The estimated error of each datum point is indicated by the size of the cross. Data from plasmids with various lengths of alternating $d(pCpG)_n$·$d(pCpG)_n$ are represented as follows: ○, pLP342 ($n = 21$); +, pLP332 ($n = 16$); △, pLP324 ($n = 12$); □, pLP316 ($n = 8$). All data shown here were taken from two-dimensional gels whose first dimension was electrophoresis in TBE buffer at room temperature. The curves drawn are calculated from the partition function described in the text.

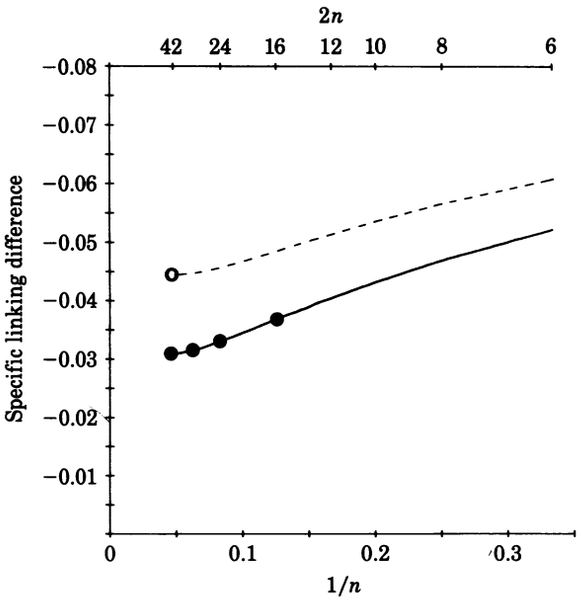

FIG. 4. Midpoints of the negative superhelicity-driven B-to-Z transition of $d(pCpG)_n$·$d(pCpG)_n$ inserts in plasmids as a function of $1/n$ (lower abscissa) or $2n$ (upper abscissa). The solid line represents the midpoints calculated from the partition function described in the text with the energy parameters determined for TBE buffer at room temperature. The filled circles are the observed transition midpoints. The broken line represents the midpoints calculated from the energy parameters estimated when 0.1 M sodium chloride is present in addition to TBE buffer. The open circle is the observed transition midpoint.

Direct Measurement of the Helical Periodicity of d(pCpG)$_n$·d(pCpG)$_n$ in the Right-Handed and Left-Handed Helical Conformations in Solution. In the above calculations, the parameter a is evaluated by taking the Z structure in solution to be the same as that of the crystal (21). The recent methods developed in this laboratory (5, 6, 22) are capable of determining the handedness and periodicity of a particular DNA sequence in solution directly. We have measured the helical periodicity of the alternating C-G sequence in the right-handed helical geometry by the band-shift method (5, 6) and the result is 10.5 ± 0.1 bp per turn in TBE buffer at room temperature (results not shown).

The determination of the helical periodicity of alternating C-G in the left-handed geometry in TBE is technically hindered because the structure is present only in plasmids that are fairly negatively supercoiled, especially for plasmids with small C-G inserts. In the high superhelicity range, however, the resolution of topoisomers by agarose gel electrophoresis is poor and precise data are difficult to obtain. We have therefore added the trivalent ion $Co(NH_3)_6^{3+}$ to the TBE buffer to facilitate the formation of the Z structure at a lower negative superhelicity (23).

Fig. 5 shows the electrophoretic patterns of mixtures of topoisomers from plasmids containing d(pCpG) inserts that have been driven into the left-handed form by the combined action of Co(III) and negative superhelicity. Two plasmids differing by four alternating d(pCpG) units, pLP324 and pLP332, were electrophoresed in lane E of Fig. 5. The longer DNA is observed to shift upwards, that is, in the direction of increasing linking number, by 0.74 times the interband spacing. This corresponds to the 8 bp residing in a left-handed helix with a helical repeat of 8/0.74 = 11 bp per turn (5, 6). In order to obtain more precise data, pairs of DNAs that differ by larger numbers of bp are used and corrections for the intrinsic length dependence of electrophoretic mobility are made. The electrophoretic patterns of several pairs of plasmids are also shown in Fig. 5, and the helical repeat of the left-handed d(pCpG)$_n$·d(pCpG)$_n$ is calculated to be 11.6 ± 0.3 bp per turn from the measured shifts (6), in close agreement with the crystal data (21).

Stabilization of Right-Handed d(pCpG)$_n$·d(pCpG)$_n$ by Small Amounts of Sodium Ion. When 0.1 M NaCl is added to the TBE buffer during electrophoresis in the first dimension, the negative superhelicity required to flip the d(pCpG)$_{21}$·d(pCpG)$_{21}$ insert to the left-handed form is increased significantly: the transition in TBE plus 0.1 M Na^+ occurs around $\alpha - \alpha_0^\circ$ of −18 rather than −12.5 (data not shown). By applying the statistical mechanical analysis described in the previous section, the energy parameters ΔG_{BZ} and ΔG_J in the presence of 0.1 M sodium are calculated to be 0.65 kcal mol^{-1} per bp and 5.0 kcal mol^{-1} per junction, respectively; the parameter b is calculated to be −0.4 turns. The broken curve shown in Fig. 4 is calculated by using these parameters, and the open circle in Fig. 4 represents the measured datum point in TBE plus 0.1 M NaCl.

It has been reported previously that the addition of NaCl at a few tenths molar to a dilute aqueous buffer destabilizes an internal Z block within a B DNA, and a higher negatively superhelicity is needed to effect the B-to-Z transition (12, 24). Our data suggest that this destabilization of the Z structure results from an increment of the free energy change ΔG_{BZ} when NaCl is added to the buffer, whereas the junction free energy change ΔG_J remains about the same. It is well known that, for poly(dCG·dCG), the left-handed helical form is the stable one in several molar salt solutions (1, 3, 25). In concentrated salt solutions, cloned alternating C-G segments also flip to the left-handed helical form at much lower negative superhelicity than that needed to effect the same transition in a low salt medium (7, 12). Thus, as the salt concentration is gradually increased to several molar, ΔG_{BZ} must first increase, pass through a maximum, and then decrease. The structural basis for this dependence of ΔG_{BZ} on salt concentration is unclear. Several authors

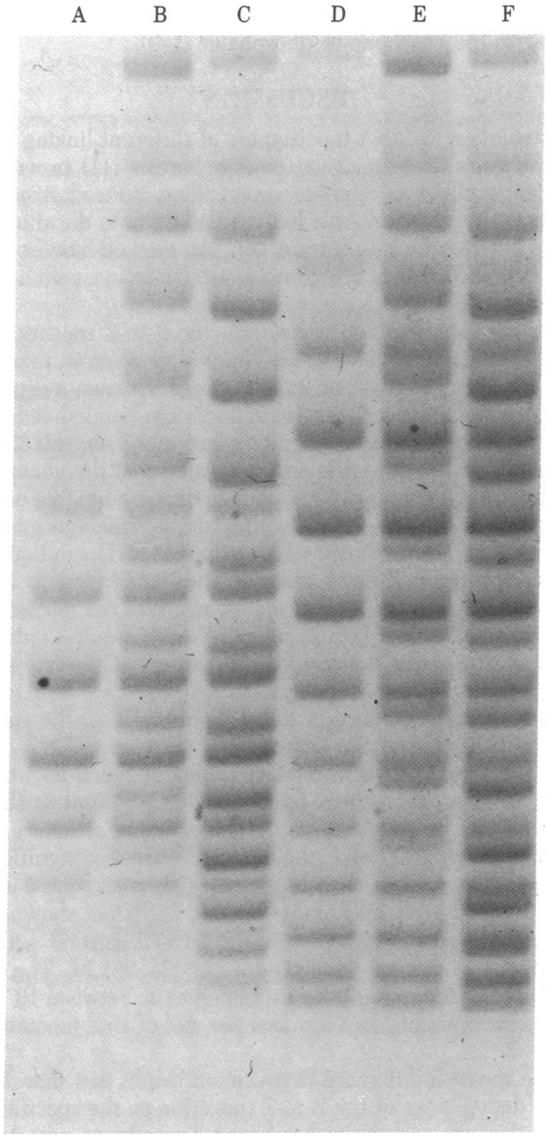

FIG. 5. Electrophoretic patterns of negatively supercoiled plasmids containing alternating d(pCpG) inserts in the left-handed form. Electrophoresis in 1.7% agarose was carried out in TBE buffer with the addition of 150 μM hexammine cobalt(III) at close to room temperature. Plasmids present in the gel lanes are A, pLP316; B, pLP316/pLP332; C, pLP316/pLP342; D, pLP324; E, pLP324/pLP332; and F, pLP324/pLP342. Calculation of the helical repeat of the alternating C-G inserts was done as before (6); the length correction term for left-handed alternating C-G is not known and that determined for right-handed DNA sequences (6) was used in all calculations. Even in the presence of hexammine Co(III) ions, the B-to-Z transition of the C-G inserts is incomplete in the less negatively supercoiled topoisomers. Calculation based on the curves shown in Fig. 3 shows, however, that due to the cancellation of errors for a pair of plasmids containing C-G inserts of different lengths, the helical repeat of the inserts calculated from the mobility differences of the pair is insensitive to incomplete transition in the C-G insert of each of the pair of plasmids. The uncertainty in the length correction term and the variation in the completeness of the B-to-Z transition in different topoisomers are responsible for the larger estimated error (±0.3) of the helical repeat determined for left-handed alternating C-G.

have pointed to the possible effects of water activity and ion binding to the backbone of phosphates (1–3).

DISCUSSION

The resolution of DNA topoisomers of different linking numbers by two-dimensional gel electrophoresis (11) provides a powerful method to examine supercoiling-induced structural changes of the DNA double helix. In addition to the study reported here for the B-to-Z transition, the method has also been applied to the study of cruciform formation from a palindromic sequence (26, 27).

The multistate model we used for the B-to-Z transition fits the experimental data well. In TBE buffer at room temperature, the intrinsic free energy difference between a d(pCpG·pCpG) unit in the left-handed Z and right-handed B helical structure is calculated to be +0.66 kcal mol^{-1}, or +0.33 kcal per mol of base pairs. It is also calculated that the generation of a B/Z junction is associated with an unfavorable free energy change of +5 kcal mol^{-1}. This unfavorable free energy is responsible for the highly cooperative transition. The initiation of a Z block within a B block generates two B/Z junctions with a free energy cost of +10 kcal mol^{-1}, whereas the lengthening of an internal Z block by 1 pCpG·pCpG unit requires only 0.66 kcal mol^{-1}. For our clones with alternating C-G inserts of 8 to 21 d(pCpG·pCpG) units, the transition from 80% B form to 80% Z form occurs in a range of a few thousandths in the specific linking difference of the DNA.

In the statistical mechanical modeling of the B-to-Z transition, we have taken the 2-bp d(pCpG·pCpG) unit as the repeating unit on the basis of crystallographic structure. The parameters that describe the transition are not significantly changed, however, if each single base pair is taken as a unit in the statistical mechanical formulation (results not shown).

This high cooperativity we observed is consistent with the sharp transition in poly(dpCpG·dpCpG) (25, 28, 29). The cooperativity parameter has been estimated to be between 10^{-4} and 10^{-6}, corresponding to a few kcal per mol of B/Z junction (28, 29).

The apparent difference between our results that show a very steep dependence of the B-to-Z transition on the specific linking difference and the rather broad dependence reported by others (12) probably results from the low resolution and the presence of two alternating C-G blocks in the plasmid used in the earlier work. One-dimensional gel electrophoresis was employed to analyze samples each containing a mixture of about eight topoisomers in the earlier work, whereas in the two-dimensional analysis each individual topoisomer is examined.

The statistical mechanical calculation reveals a rather large unwinding associated with each B/Z junction. The best estimate of this unwinding is about −0.4 turn at each junction. Attempts to fit the data points with less negative values of b give poorer fits. That there appears to be a substantial unwinding at the B/Z junction can also be seen by inspection of Fig. 3. The maximal curve value of $\langle \Delta Tw \rangle$ approaches a value significantly larger than the $-n[(1/10.5) + (1/12)]$ turns expected when a 10.5-bp per turn B helix flips to a 12-bp per turn Z helix.

Several model building studies give somewhat variable structural features for the B/Z junction (21, 30). It appears from these studies, however, that an unwinding of near one-half of a turn at each junction is larger than expected. Interestingly, it has been shown that the B/Z junction is sensitive to cleavage by the single-strand-specific nuclease S1 (12). Although it is not possible to quantitate the nuclease S1 results in terms of the magnitude of unwinding at the junction, both observations indicate significant destabilization of the DNA double helix at a B/Z junction.

Finally, our band-shift experiment has confirmed that the helical hand of an internal alternating C-G sequence in a relaxed DNA is indeed changed when the DNA is sufficiently negatively supercoiled. The periodicity of the negative supercoiling-induced left-handed helical structure in solution, 11.6 ± 0.3 bp per turn, agrees with that of the Z structure in the crystal (21).

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